Scalpellum
JOURNAL OF SURGERY & CLINICAL RESEARCH

Original Article

BHLHE22 Expression in Breast Cancer: a TCGA Database Analysis

Nurul Azizah[1], Oktri Yetta Wimarti[2], Soraya[3], Suyanto[4], Stephani Browlim[4] and Darmawi[5*]

[1]Departement Biochemistry, Faculty of Medicine, Muhammadiyah University of Riau, Pekanbaru, Indonesia;
[2]Master Program in Biomedicine, Faculty of Medicine, Universitas Riau, Pekanbaru, Indonesia;
[3]Departement Biochemistry, Faculty of Medicine, Awal Bross University, Pekanbaru, Indonesia;
[4]Departement of Public Health; [4]Bachelor Program in Medicine;
[5]Departement of Histology, Faculty of Medicine, Universitas Riau, Pekanbaru, Indonesia.

*Corresponding author: darmawi@lecturer.unri.ac.id

Abstract

Breast cancer (BC), an estrogen-dependent malignancy, hinge on the mitogenic outcome of estrogen in multiplying tumorigenesis and tumor growth. BC reported as one of the most common and death caused cancer in female. The basic helix-loop-helix family member e22 (BHLHE22), an intronless gene is critical regulator for genes expression and have been linked to estrogen-dependent type of endometrial cancer. The aim of this study is to comprehensively analyze the expression of BHLHE22 in breast cancer patients from TCGA database. BHLHE22 genotype data and clinical information about BC patients were obtained using Cbiom, XENA, and ATLAS data from the TCGA breast cancer database. The R studio and Medcalc software was used to analyze statistical data and create graphs. We found that BHLHE22 is a breast cancer survival and immunology-related gene. Different associations between BHLHE22 and survival were identified in this investigation, and it was demonstrated that BHLHE22 gene expression increased in radiotherapy-treated breast cancer patients. BHLHE22 is an immune system gene, and the link between it and the BHLHE22 gene decreases in malignancies at an advanced stage. Therefore, in order to advance research on breast cancer patients, it is vital to consider ways to improve the immune systems of breast cancer patients by examining the specific function of the BHLHE22 gene. BHLHE22 has the ability to influence immune-related pathways and the immunological status of the BC microenvironment stage. Therefore, in order to advance research on breast cancer patients, it is vital to consider ways to improve the immune systems of breast cancer patients by examining the specific function of the BHLHE22 gene. More research is required on the immunomodulatory role of BHLHE22 in cancer.

Keywords: BHLHE22, breast cancer, immunomodulatory, survival, TCGA

OPEN ACCESS
Submitted: 30 May 2025
Revised: 14 June 2025
Accepted: 20 June 2025
Published: 30 June 2025

Corresponding author
Darmawi
darmawi@lecturer.unri.ac.id

Academic editor
Fajri Marindra

Data Availability Statement
All relevant data are within the paper and its Supporting Information files.

Introduction

Breast cancer is a malignancy in the breast tissue. Breast tissue consists of connective tissue, fatty tissue, mammary glands, and the ducts of the mammary glands. Normally, old and dead breast cells are replaced with new ones, but in breast cancer, the genes responsible for cell growth undergo mutations so that

breast cells proliferate non-stop and become malignant.[1]

Breast cancer ranks fifth in cancer-related mortality with 684,996 deaths (6.9%) and is the most frequent cancer globally with 2,261,419 new cases (11.7%), according to GLOBOCAN 2020 data.[2,3] In the United States, SEER 2022 data reported breast cancer as the leading cause of new cancer cases and deaths among women, with 287,850 new cases (15%) and 43,250 deaths (7.1%).[4] Regionally, Asia accounts for the highest incidence (45.4%) and mortality (50.5%), followed by Europe (23.5% incidence, 20.7% mortality).[2] The rising incidence in developing countries is linked to urbanization and Western lifestyle adoption.[5] In Indonesia, breast cancer ranks first among malignancies, accounting for 16.6% of all cancers and 30.8% of cancers in women.[2]

Early detection through screening mammography, along with advancements in surgical procedures and adjuvant treatment, has improved the five-year survival rate for women with breast cancer from 63% in 1960 to 90% in 2018 (American Cancer Society).[6] According to the United States End Results and Surveillance Epidemiology (SEER), 90.6% of patients with breast cancer survived for five years between 2012 and 2018.[4]

The development of breast cancer (BC) is significantly influenced by the tumour microenvironment. Numerous preclinical studies have determined that the immunological environment, stromal cells, and inflammation are significant contributors to BC.[7] The prognosis for BC, a type of cancer that advances quickly, is poor, and the TME can significantly affect how the illness develops. Important elements of the tumour microenvironment (TME), immune and stromal cells can significantly impact tumour development, proliferation, and response to treatment.[8] The evidence that TME promotes BC development, invasion, and metastasis is growing. Although immune cells and soluble chemicals alter the progression of cancer, TME-related genes and the prognosis of BC are unknown. In BC, a large number of cells are classified as immune cells based on both stromal and immune cell infiltration. They include BHLHE22.[7]

Out of the roughly 600 gene families in BHLH, only 15% of those pertaining to humans, animals, and plants may be published. Certain environmental elements, such as glucose, sterols, inositol, choline, iron, and calcium, can cause cells to express alternative genes in response. They can also support cellular and systemic defence against hypoxia and xenobiotics, as well as synchronize organismal growth in response to light. The variety of cell types during embryogenesis necessitates the addition of new members through cell-type-specific differentiation, proliferation, death, and determination.[9] Important regulators of cell proliferation, differentiation, and fate determination are proteins that belong to the basic helix-loop-helix (BHLH) family.[1020] As ubiquitous family members, they often behave as tissue-specific heterodimers that bind to a common E-box sequence.[19]

BHLHE22, like other BHLH families, is known to have a role in neuron and cell development, transcriptional repression, and transcription factor activity (19 and 21). Regulatory T cells, myeloid dendritic cells, resting myeloid dendritic cells, B cells, M1 macrophages, CD8+, CD4+, and memory CD4+ T cells are among the immune cells that have also been linked to BHLHE22 for invading the tumour microenvironment. Stimulated myeloid dendritic cells with negatively correlated BHLHE22 expression had higher survival rates. In brain, lung, breast, cervix uteri, colon, and skin cancers, BHLHE22 is downregulated, which suggests a widespread role in cancer biology.

There hasn't been much focus on BHLHE22's impact on cancer clinical outcomes. BHLHE22 is a signature gene associated with immune responses and survival, according to a recent study on breast cancer.[7,20]

Methods

BHLHE22 genotype data and clinical information about BC patients were obtained using Cbiom, XENA, and ATLAS data from the TCGA breast cancer database. A total of 1,248 BC samples (combined set) were used to create training and validation sets of varying sizes. The validation set is used to verify the training set's findings. Since all of the data utilized in this study came from publicly accessible sources, ethical approval is not needed. This study was conducted in compliance with the 2013 edition of the Helsinki Declaration. R Studio and Medcalc were used to create graphs and evaluate statistical data. We employed a number of statistical tests, including the T-test. For all values, the mean standard deviation (SD) is used. A value of $P < 0.05$ was considered statistically significant. The prognosis was then examined using a histogram graph for each gene. Then we looked at the prognosis via a Histogram graph for each gene. BHLHE22 expression levels were divided into “high” and “low” expression groups based on the median value of expression across all breast cancer samples. The high expression group was defined as patients whose expression levels were higher than the median, and the low expression group as those whose expression levels were lower than the median. The median split method was employed to make group comparisons easier.

Results

Differences and Corelation Data with BHLHE22 gene.

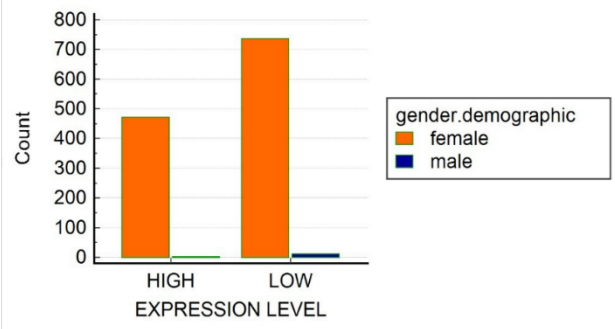

Figure 1. Differences in Expression level gen BHLHE22 score in gender (female and male) Breast Cancer Patient.

In **Figure 1**, there is a difference between the expression levels of male and female breast cancer patients. where the female expression level is greater than the male expression level. Several other clinical characteristics exhibit variations in the expression of this level, as shown in the **Table 2**.

Table 1. Differences in Expression level gen BHLHE22 score in vital status (Alive and Dead) Breast Cancer Patient.

	High Expression	Low Expression	Total	p-value
Alive	388	1018	1406	0.0208*
Dead	117	202	319	
Total	505	1220	1725	

*Significant α 0.05

The association between 1,725 breast cancer patient's vital status (living vs. dead) and BHLHE22 gene expression levels (high vs. low) was investigated using a chi-square test, as shown in **Table 1.** Of those with high expression, 388 were still alive and 117 had passed away, while 1,018 had low expression and were still alive and 202 had passed away. Compared to patients in the low expression group (16.6%), patients in the high expression group had higher death rates (23.2%). A statistically significant correlation between survival status and gene expression level (α = 0.05) is indicated by the resulting p-value of 0.0208, indicating a substantial relationship between vital outcomes and BHLHE22 expression. This confirms that the distribution of alive and dead patients differs meaningfully across gene expression levels, supporting the validity of the data analysis.

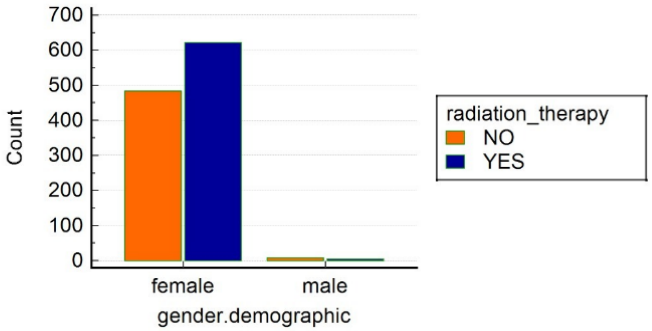

Figure 3. Correlations between Radiation Therapy and Gender Demographic (female and male).

With reference to **Figure 3**, it depicts the gender-demographic distribution of patients who got radiation therapy (YES) and those who did not (NO). The data clearly show that female patients are far more represented and are more likely to have undergone radiation therapy compared to male patients. However, this distribution may also reflect the general prevalence of breast cancer being significantly higher in females than in males. After the data was processed using Medcalc and R studio software, the characteristics of breast cancer patients were obtained based on age, sex, radiation therapy, tumor stage, tumor histology, and patient's vital status. Based on the data obtained, the age range of most breast cancer patients is in the range of 56-65 years and high BHLH2 gene expression is also present in this age range. The highest sex was female, recorded 99%, with the lowest BHLH22 gene expression level being the highest. More patients had received radiation therapy than those who did not as much as 49% of the total, with most of them being patients with low BHLHE22 gene expression levels. The majority of individuals with early-stage breast cancer (73 percent of all cases) had elevated BHLHE22 gene expression. For the most part, the tumour's histological characteristics are uncertain or unknown (NA). Even with increased levels of BHLHE22 gene expression, more patients with breast cancer survived, up to 83% in vital status. **Table 1** lists the attributes of patients with breast cancer.

Discussion

When expressed in specific cell types, BHLH proteins activate several genes and specific phenotypes. The BHLH domain consists of a basic region for binding to a target DNA sequence and a helix-loop-helix (HLH) motif, which is composed of two a-helices separated by a loop of variable length. Protein architectures, dimerization selectivity, expression patterns, molecular phylogenetic relationships, and DNA-binding specificities have all been used to classify BHLHs into distinct groups.[24,25]

Table 2. Characteristics of breast cancer patients

Number	Clinical Characteristic	Classification	Frequency %	Expression HIGH	Expression LOW
1	Age	16-25	0%	0	0
		36-35	3%	11	21
		36-45	13%	66	76
		46-55	25%	125	150
		56-65	29%	128	191
		66-75	17%	56	129
		76-85	10%	33	82
		86-95	2%	9	18
		NA	2%	5	12
2	Gender	Male	1%	2	11
		Female	99%	471	736
		NA	0%	1	0
3	Radiation Therapy	Yes	49%	251	346
		No.	38%	156	306
		NA	13%	67	95
4	Tumor Stage	Early	73%	562	334
		Advance	25%	170	132
		NA	2%	15	7
5	Histological Type	Available	7%	53	33
		NA	93%	694	441
6	Vital Status	Alive	83%	630	388
		Dead	17%	117	85
		NA	0%	0	1

It has been shown that BHLHE22 is connected to cancer, neuropathies, and developmental disorders in addition to neuro-specific activity. Intronless genes are crucial regulators of regulatory processes.[26] BHLHE22 is implicated in cell differentiation by regulating Cadherin-11 and building a repressor complex with Prdm8, according to a study on brain development.[19,21] Additional BHLH family members, particularly those related to brain tumours such as ASCL1 in glioma and neuroblastoma, Olig2 in astrocytoma, and ATOH1 in medulloblastoma, have been documented in cancer biology and clinical outcomes.[7] Nevertheless, it is uncommon to find reports of BHLHE22 with EC clinical features. Limited research has been done on the role of BHLHE22 in cancer clinical outcomes. A recent study on breast cancer found that BHLHE22 is a hallmark gene that is tightly linked to immune responses and survival.[27]

In endometrial cancer, BHLHE22 is thought to be related to the immune system. Endometrial cancer BHLHE22 expression level and leukocyte infiltration revealed positive findings. Immune cells that infiltrated the tumour microenvironment, such as B cells, M1 macrophages, regulatory T cells, myeloid dendritic cells, resting myeloid dendritic cells, CD8+ T cells, CD4+ T cells, and activated memory CD4+ T cells, were significantly positively correlated with BHLHE22 expression, while activated myeloid dendritic cells were negatively correlated.[20] The gene BHLHE22 is linked to immunology and breast cancer survival. BHLHE22 is one of the potential therapeutic targets for breast cancer.[7] In this study, many associations between BHLHE22 and survival were discovered, and it was demonstrated that patients with breast cancer who received radiation therapy had increased BHLHE22 gene expression.

The most often diagnosed malignancy is breast cancer. In 2020, 2.3 million new cases will occur worldwide.[28] The authors attempt to provide a table that summarizes the various traits associated with breast cancer. Breast cancer cases are displayed in **Table 1** according to the patient's vital status, age, sex, radiation therapy, tumour stage, and histology. The majority of breast cancer patients, as indicated by **Table 1**, were between the ages of 56 and 65 and had

the highest levels of BHLHE22 gene expression, specifically above the median value of 13,625. This is in line with data showing that older women, specifically those who have gone through menopause, have a higher incidence of breast cancer. In 2018, there were up to 1.4 million new cases in postmenopausal women, compared to just 645,000 new cases in premenopausal women, and 490,000 deaths in postmenopausal women and 130,000 deaths in young women.[28] According to **Table 1**, breast cancer is more likely in women (99%), who also had lower BHLHE22 gene expression. In 2020, according to WHO data, breast cancer ranks first among cancers that harm women, while prostate cancer is the most frequent cancer among men.[29] Endotherapy, chemotherapy, radiation, surgery, and immunotherapy are some of the treatments used to treat breast cancer.[30,31]

Radiation therapy was used to treat 49% of breast cancer cases, according to sample data from individuals with the disease that the authors collected for Cbiom. Advances in sequencing methods, next-generation sequencing, spatial gene profiling, and enhanced bioinformatics support are some of the current developments in the detection of breast cancer.[32-34] The early detection of breast cancers is made possible by this. According to **Table 1**, a higher percentage of patients—73%—have been diagnosed with early-stage breast cancer. Subtypes of breast cancer differ in their molecular profiles and histological morphological characteristics.[28,35]

This is consistent with the information in **Table 1**, which shows that there are 93% more NA histological preparations than there are currently available. Because of its diversity, NA may indicate that a histological investigation was not performed, that it was not classified, or that it was not characterized. Moreover, the histological and clinical characteristics of this breast cancer vary depending on the patient's age and ethnicity.[36-38] In 2018, the International Agency for Research on Cancer (IARC) reported a 6.9% breast cancer mortality rate and an 11.7% new case rate based on GLOBOCAN data. This indicates that there have been a significant number of fatalities.[28] According to the data in **Table 1**, 17% of patients passed away, which was less than the 83% of patients who survived.

Data with statistically significant results are included in this review. The age range data shows that the largest age range is 86–95 years old, while the smallest is 16–25 years old. The BHLHE22 gene's expression level was then linked to this age range, and the results showed statistical significance with a p-value of less than 0.0001. Likewise, the connection between radiation therapy and BHLHE22 gene expression There are two categories of people: those who receive radiation therapy and those who do not. Significant results are indicated by the obtained results' P value of 0.000061. The BHLHE22 gene's expression level, on the other hand, was not substantially correlated with vital state, histological type, tumour stage, or gender. BHLHE22 expression was higher in the microsatellite-instable subtype, endometrioid type, grade, and age. Survived well. Overexpression of BHLHE22 inhibited EC cell growth and migration. BHLHE22's immune-related pathways were considerably enriched. BHLHE22 also increased EC chemokine gene expression and proinflammatory leukocyte infiltration. BHLHE22 controls immunological pathways and the EC's immune microenvironment.[20]

The pathogenesis of the BHLHE22 gene causing breast cancer is not fully clear, but it is likely related to the function of the BHLHE22 gene as a cell transcription suppressor and involved in cell differentiation. Very little research and literature on the BHLHE22 gene related to breast cancer, in contrast to the case of endometrial cancer. In endometrial cancer it was found that the BHLHE22 gene was hypermethylated in cancer tissue, this can be identified with a Pap-smear test. BHLHE22 expression that is too high or too low can prevent endometrial cancer cells from migrating and proliferating. The immunological microenvironment of cancer is likewise modulated by BHLHE22, which also controls immune-related pathways.[20]

In **Table 1**, the authors' sample data from Siena showed that 83% of breast cancer patients survived, had greater BHLHE22 gene expression levels in the high group, and had more early-stage diagnoses. This demonstrates that while breast cancer still accounts for the largest percentage of malignancies, it is now better managed. The five-year survival rate for women with breast cancer has increased from 63% in 1960 to 90% in 2018, according to the American Cancer Society, because to early identification by screening mammography, improvements in surgical techniques, and adjuvant treatment.[39] According to Surveillance Epidemiology and The End Results (SEER), people with breast cancer had a 90.6% five-year survival rate between 2012 and 2018.[40] Moreover, new technologies are always being created, like sequencing methods for detecting gene abnormalities linked to cancer and other bioinformatics assistance. Patients with breast cancer have a better chance of surviving as a result. Drawing from the data we have linked the BHLHE22 gene to the vital status or survival of breast cancer patients, we discovered that the expression of the BHLHE22 gene was lower in patients with advanced-stage tumours and greater in patients with breast

cancer who were still living. This finding is based on data that links the BHLHE22 gene to breast cancer patients' survival.

For cell differentiation to occur, the basic helix loop helix transcription factor family, BHLHE22, must be transcriptionally repressed. It is uncertain how BHLHE22 contributes to EC. Using databases (TCGA, GTEx, and the human protein atlas), the expression of BHLHE22 was analyzed in 54 samples of endometrial tissue, both healthy and malignant. In comparison to normal endometrium, EC showed considerably decreased levels of BHLHE22 protein expression. Though it is thought that breast cancer can be examined, the role of the BHLHE22 gene in breast cancer has not been investigated. Breast cancer kills. Thus, BHLHE22 gene studies can reduce cell damage and infection.[20] Because BHLHE22 is an immune system gene, the correlation between it and the BHLHE22 gene also declines in advanced-stage malignancies. Therefore, in order to further research on breast cancer patients, it is essential to think about how to enhance breast cancer patients' immune systems by looking back at the unique role of the BHLHE22 gene. This is crucial since breast cancer patients often have a weakened immune system.

Conclusion

The immunological status of the BC microenvironment and immune-related pathways may be altered by BHLHE22. Further investigation is required on the immune-modulatory role of BHLHE22 in BC.

Ethics approval

The Ethical approval is not required.

Acknowledgments

All authors equally contributed to case identification, manuscript drafting, and revision.

Competing interests

All the authors declare that there are no conflicts of interest.

Funding

This study received no external funding.

Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

Declaration of artificial intelligence use

We hereby confirm that no artificial intelligence (AI) tools or methodologies were utilized at any stage of this study, including during data collection, analysis, visualization, or manuscript preparation. All work presented in this study was conducted manually by the authors without the assistance of AI-based tools or systems.

References

1. Mardiana L. Kanker pada wanita. Jakarta: Niaga Swadaya; 2007.
2. National Cancer Institute. Surveillance Epidemiology and End Results (SEER). 2022. Available from: https://seer.cancer.gov/statfacts/
3. Miller KD, Nogueira L, Devasia T, Mariotto AB, Yabroff KR, Jemal A, et al. Cancer treatment and survivorship statistics, 2022. CA Cancer J Clin. 2022;72(5):409-36.
4. Porter P. "Westernizing" women's risks? Breast cancer in lower-income countries. N Engl J Med. 2008;358(3):213-6.
5. Cancer Statistics Center. Breast cancer. 2018. Available from: https://cancerstatisticscenter.cancer.org/#!/cancer-site/Breast
6. Zhu J, Shen Y, Wang L, Qiao J, Zhao Y, Wang Q. A novel 12-gene prognostic signature in breast cancer based on the tumor microenvironment. Ann Transl Med. 2022;10(3).
7. Sturm G, Finotello F, Petitprez F, Zhang JD, Baumbach J, Fridman WH, et al. Comprehensive evaluation of transcriptome-based cell-type quantification methods for immuno-oncology. Bioinformatics. 2019;35(14):i436-45.
8. Ledent V, Paquet O, Vervoort M. Phylogenetic analysis of the human basic helix-loop-helix proteins. Genome Biol. 2002;3(6):1-18.
9. Cserjesi P, Brown D, Ligon KL, Lyons GE, Copeland NG, Gilbert DJ, et al. Scleraxis: a basic helix-loop-helix protein that prefigures skeletal formation during mouse embryogenesis. Development. 1995;121(4):1099-110.
10. Guillemot F, Lo LC, Johnson JE, Auerbach A, Anderson DJ, Joyner AL. Mammalian achaete-scute homolog 1 is required for the early development of olfactory and autonomic neurons. Cell. 1993;75(3):463-76.
11. Jan YN, Jan LY. HLH proteins, fly neurogenesis, and vertebrate myogenesis. Cell. 1993;75(5):827-30.
12. Li L, Cserjesi P, Olson EN. Dermo-1: a novel twist-related bHLH protein expressed in the developing dermis. Dev Biol. 1995;172(1):280-92.
13. Olson EN, Klein WH. bHLH factors in muscle development: dead lines and commitments, what to leave in and what to leave out. Genes Dev. 1994;8(1):1-8.
14. Overbeek PA. Right and left go dHAND and eHAND. Nat Genet. 1997;16(2):119-21.
15. Porcher C, Swat W, Rockwell K, Fujiwara Y, Alt FW, Orkin SH. The T cell leukemia oncoprotein SCL/tal-1 is essential for development of all hematopoietic lineages. Cell. 1996;86(1):47-57.
16. Srivastava D, Cserjesi P, Olson EN. A subclass of bHLH proteins required for cardiac morphogenesis. Science. 1995;270(5244):1995-9.
17. Weintraub H. The MyoD family and myogenesis: redundancy, networks, and thresholds. Cell. 1993;75(7):1241-4.
18. Xu ZP, Dutra A, Stellrecht CM, Wu C, Piatigorsky J, Saunders GF. Functional and structural characterization of the human gene BHLHB5, encoding a basic helix-loop-helix transcription factor. Genomics. 2002;80(3):311-8.

19. Darmawi, Chen LY, Su PH, Liew PL, Wang HC, Weng YC, et al. BHLHE22 Expression Is Associated with a Proinflammatory Immune Microenvironment and Confers a Favorable Prognosis in Endometrial Cancer. Int J Mol Sci. 2022;23(13).
20. Ross SE, McCord AE, Jung C, Atan D, Mok SI, Hemberg M, et al. Bhlhb5 and Prdm8 form a repressor complex involved in neuronal circuit assembly. Neuron. 2012;73(2):292-303.
21. Murre C, Bain G, van Dijk MA, Engel I, Furnari BA, Massari ME, et al. Structure and function of helix-loop-helix proteins. Biochim Biophys Acta. 1994;1218(2):129-35.
22. Massari ME, Murre C. Helix-loop-helix proteins: regulators of transcription in eucaryotic organisms. Mol Cell Biol. 2000;20(2):429-40.
23. Atchley WR, Fitch WM. A natural classification of the basic helix-loop-helix class of transcription factors. Proc Natl Acad Sci U S A. 1997;94(10):5172-6.
24. Ledent V, Vervoort M. The basic helix-loop-helix protein family: comparative genomics and phylogenetic analysis. Genome Res. 2001;11(5):754-70.
25. Aviña-Padilla K, Ramírez-Rafael JA, Herrera-Oropeza GE, Muley VY, Valdivia DI, Díaz-Valenzuela E, et al. Evolutionary perspective and expression analysis of intronless genes highlight the conservation of their regulatory role. Front Genet. 2021;12:654256.
26. Dennis DJ, Han S, Schuurmans C. bHLH transcription factors in neural development, disease, and reprogramming. Brain Res. 2019;1705:48-65.
27. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394-424.
28. International Agency for Research on Cancer. GLOBOCAN: Global Cancer Observatory. 2020. Available from: https://gco.iarc.fr/today/about
29. Nounou MI, ElAmrawy F, Ahmed N, Abdelraouf K, Goda S, Syed-Sha-Qhattal H. Breast cancer: conventional diagnosis and treatment modalities and recent patents and technologies. Breast Cancer (Auckl). 2015;9:BCBCR.S29420.
30. Sharma GN, Dave R, Sanadya J, Sharma P, Sharma K. Various types and management of breast cancer: an overview. J Adv Pharm Technol Res. 2010;1(2):109-15.
31. Russnes HG, Navin N, Hicks J, Borresen-Dale AL. Insight into the heterogeneity of breast cancer through next-generation sequencing. J Clin Invest. 2011;121(10):3810-8.
32. Zhang J, Späth SS, Marjani SL, Zhang W, Pan X. Characterization of cancer genomic heterogeneity by next-generation sequencing advances precision medicine in cancer treatment. Precis Clin Med. 2018;1(1):29-48.
33. Fumagalli C, Ranghiero A, Gandini S, Corso F, Taormina S, De Camilli E, et al. Inter-tumor genomic heterogeneity of breast cancers: comprehensive genomic profile of primary early breast cancers and relapses. Breast Cancer Res. 2020;22(1):1-11.
34. Ghoncheh M, Pournamdar Z, Salehiniya H. Incidence and mortality and epidemiology of breast cancer in the world. Asian Pac J Cancer Prev. 2016;17(S3):43-6.
35. Yersal O, Barutca S. Biological subtypes of breast cancer: Prognostic and therapeutic implications. World J Clin Oncol. 2014;5(3):412-24.
36. Molnár IA, Molnár BÁ, Vízkeleti L, Fekete K, Tamás J, Deák P, et al. Breast carcinoma subtypes show different patterns of metastatic behavior. Virchows Arch. 2017;470(3):275-83.
37. Rossing M, Pedersen CB, Tvedskov T, Vejborg I, Talman ML, Olsen LR, et al. Clinical implications of intrinsic molecular subtypes of breast cancer for sentinel node status. Sci Rep. 2021;11(1):1-12.
38. Cancer Statistics Center. Breast cancer. 2018. Available from: https://cancerstatisticscenter.cancer.org/#!/cancer-site/Breast
39. National Cancer Institute. SEER Cancer Statistics. 2022. Available from: https://seer.cancer.gov/statfacts/
40. Institute NC. https://seer.cancer.gov/statfacts/. Surveillance Epidemiology and End Results (SEER).